

Docket No.: 0010-1057-0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :
YOSHIHIRO USUDA ET AL : EXAMINER: FRONDA, C. L.
SERIAL NO. : 09/441,055 :
FILED: NOVEMBER 16, 1999 : GROUP ART UNIT: 1652
FOR: METHOD FOR PRODUCING L-METHIONINE BY FERMENTATION

APPEAL BRIEF

COMMISSIONER FOR PATENTS
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SIR:

This is an appeal of Claims 31, 35, and 41-49 in the above-identified application and the rejections set forth in the Official Action mailed February 7, 2008.

I. Real Party of Interest

The real party of interest is Ajinomoto Co., Inc., by virtue of the assignment recorded in the U.S. Patent and Trademark Office on February 16, 2000, at reel 010614, frames 0980-0982.

II. Related Appeals and Interferences

Appellants, Appellants' legal representative and their assignee are not aware of any appeals or interferences which will directly affect or be directly affected by or having a bearing on the Board's decision in this appeal.

III. Status of Claims

Claims 31, 35, and 41-59 are the only claims pending in the above-identified application and appear in the attached Claims Appendix. All other claims, whether original or added during prosecution, were canceled during prosecution of this application.

Claims 31, 35, and 41-59 stand rejected.

Claims 31, 35, and 41-59 are appealed herein.

IV. Status of Amendments filed under 37 C.F.R. §1.116

An Amendment under 37 C.F.R. §1.116 was not filed. Appellants now appeal the rejections set forth in the final Office Action mailed February 7, 2008.

V. Summary of the Claimed Subject Matter

As recited in independent Claim 31, the present invention provides a method for producing L-methionine which comprises culturing a recombinant *Escherichia* bacterium in a medium to produce and accumulate L-methionine in the medium, and collecting the L-methionine from the medium, wherein

the bacterium is deficient in repressor of L-methionine biosynthesis system encoded by the endogenous *metJ* gene and has L-methionine productivity,

activity of intracellular homoserine transsuccinylase encoded by the *metA* gene of a *Escherichia* bacterium is increased compared to an unmodified *Escherichia* bacterium by increasing copy number of the *metA* gene including its own promoter, or replacing the native promoter with a stronger promoter, and

the bacterium comprises at least one characteristic selected from the group consisting of:

(a) exhibits reduced activity of intracellular S-adenosylmethionine synthetase encoded by the endogenous *metK* gene as compared to an unmodified *Escherichia* bacterium;

(b) exhibits L-threonine auxotrophy;

(c) exhibits enhanced activity of intracellular cystathionine γ -synthase encoded by the *metB* gene of a *Escherichia* bacterium and enhanced activity of intracellular aspartokinase-homoserine dehydrogenase II encoded by the *metL* gene of a *Escherichia* bacterium as compared to an unmodified *Escherichia* bacterium by increasing copy number of each of the genes including their own promoters, or replacing the native promoter with a stronger promoter; and

(d) has a homoserine transsuccinylase for which concerted inhibition by L-methionine and S-adenosylmethionine is desensitized, wherein the homoserine transsuccinylase comprising the amino acid sequence of SEQ ID NO: 26 contains at least one amino acid replacement wherein said at least one amino acid replacement is independently selected from the group consisting of replacement of the amino acid residue Arg-27 with cysteine, replacement of the amino acid residue Ile-296 with serine, and replacement of the amino acid residue Pro-298 with leucine. (see the specification, at least, at page 5, line 3 to page 7, line 9, page 7, line 20 to page 8, line 6, page 8, line 12 to page 10, line 4, page 10, line 19 to page 15, line 3, page 15, lines 3-5, 9-12, and 16-21, page 16, line 8 to page 20, line 7, and page 41, lines 8-17)

Appellants note that Claims 41-55 require that one of characteristics (a)-(d) defined in Claim 31 or a combination thereof be present, while Claims 58-59 define the mutation resulting in L-threonine auxotrophy. Claims 56-57 further define the mutation resulting in reduced activity of intracellular S-adenosylmethionine synthetase. These limitations find support, at least, in the cited sections above.

VI. Grounds of Rejection to be Reviewed on Appeal

1. Claims 31, 35, and 41-59 stand rejected under 35 U.S.C. §103(a) over Michaeli et al¹ in view of the combined disclosures of Greene² and Park et al³.

VII. Arguments

- (A) Claims 31, 35, and 41-59 stand rejected under 35 U.S.C. §103(a) as being obvious over Michaeli et al in view of the combined disclosures of Greene and Park et al.

This rejection is untenable and should not be sustained.

Claims 31 and 35 are rejected as being unpatentable over Michaeli et al, in view of the combined teachings of Greene and Park et al. In the Office Action, the Examiner recognizes that Michaeli et al differs from the claimed invention, at least, in that this reference does not teach a recombinant *Escherichia* bacterium deficient in the metJ gene encoding a repressor of the L-methionine biosynthesis gene. The Examiner alleges that Greene teach the E. coli repressor of the L-methionine biosynthesis system encoded by the metJ gene. The Examiner further alleges that Park et al teach the enzyme *E. coli* metK gene encoding S-adenosylmethionine synthetase which catalyzes the synthesis of S-adenosyl-L-methionine (SAM), where SAM is a major methyl group transfer agent in biological systems and the methyl moiety of SAM is transferred to proteins, lipids, nucleic acids, and vitamins by SAM-dependent methyltransferases.

However, in Park et al, the central theme is the enzymatic synthesis of SAM using S-adenosylmethionine synthetase encoded by the metK gene. This enzyme is subject to product

¹ Advances in Polyamine Research (1983), 4, 519-520.

² *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, 2nd Ed., pps. 542-560, "Biosynthesis of Methionine", 1996.

³ Bioorg. Med. Chem., 1996 Dec; 4(12):2179-2185.

inhibition. To avoid the problem, Park et al searched additives which overcome product inhibition of the enzyme as summarized in Table 1. Thus, an object and standpoint of Park et al are completely different from those of the present invention or other cited references.

What is missing in Park et al, and the other cited references, is that it does not disclose or suggest disruption of the metK gene and application of this gene to breeding of L-methionine producing strains. At best, the Examiner's case can be summarized as that the in view of the relied upon references the artisan would have the capabilities to practice the claimed invention. However, it is well settled that whether the claimed invention is within the capabilities of one of ordinary skill in the art is not sufficient by itself to establish *prima facie* obviousness (MPEP §2143.01). Indeed, the mere fact that the references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references. *Ex parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993).

In the present case, looking at the disclosure of Park et al to determine whether this reference actually provides a disclosure of disruption of the metK gene and application of this gene to breeding of L-methionine producing strains, the artisan sees that Park et al disclose that S-adenosylmethionine synthetase is subject to product inhibition. Since increased product formation would shut down the enzymatic activity, the skilled artisan would not be motivated to disrupt the metK gene absent a specific disclosure that L-methionine producing strains do not suffer from the above-mentioned product inhibition, or the inhibition is desensitized in L-methionine producing strains. Such a disclosure does not exist in the cited art. Accordingly, Appellants submit that even if the artisan were to have Park et al in hand,

the combined disclosures of this reference with Michaeli et al and Greene would not be sufficient to render the claimed invention even *prima facie* obvious.

Moreover, Appellants submit that absent the present application, the artisan would not have been led to the L-methionine production ability of the claimed method. Specifically, reference is made to Example 3, where a metK mutation and metA amplification are performed on various L-threonine auxotrophic strains (W Δ BC; see Example 1) and metJ deficient strains (W Δ J and W Δ BC Δ J; see Example 1). What is clear from the Table on page 39 of the specification is that introduction of metA amplification into the strains that are either various L-threonine auxotrophic strains or metJ deficient strains provide enhanced production of methionine as compared to amplification of metA in *E. coli* W3110, a derivative of the wild-type K-12 strain of *E. coli* (see page 26, line 4-6). The production of methionine is further enhanced by the additional mutation of metK to reduce the activity of intracellular S-adenosylmethionine synthetase encoded thereby (see the last two rows of the table on page 39). Absent the present specification, this enhanced production of methionine would not have been apparent.

Appellants further note that Claims 42-55 require that one of characteristics (b)-(d) defined in Claim 31 or a combination thereof be present in the claimed method with or without characteristic (a). The Examiner rejects these claims; however, at no point does the Examiner provide any attempt to provide a basis for rejecting the same or explain how these specific limitations would be “obvious” in view of the cited references, which is contrary to 37 CFR 1.104(c). Indeed, there is a good reason for the Examiner’s omission of an explanation or basis for rejection of Claims 42-55. Specifically, none of Michaeli et al, Greene, or Park et al disclose or suggest characteristics (b)-(d). Appellants submit that the rejection over Claims 42-55 should be withdrawn and these claims passed to allowance.

Similarly, the Examiner's rejection fails to offer any explanation and/or basis for rejecting Claims 58-59, which define the mutation resulting in L-threonine auxotrophy (characteristic (b)), or Claims 56-57, which further define the mutation resulting in reduced activity of intracellular S-adenosylmethionine synthetase). Again, the blanket rejection without any basis or explanation of the pertinence of the cited references is contrary to 37 CFR 1.104(c). As was the case with Claims 42-55, there is a good reason for the Examiner's omission of an explanation or basis for rejection of Claims 56-59. Specifically, none of Michaeli et al, Greene, or Park et al disclose or suggest the limitations claimed therein. Appellants submit that the rejection over Claims 56-59 should be withdrawn and these claims passed to allowance.

Accordingly, it is respectfully requested that this rejection be REVERSED.

VIII. CONCLUSION

For the above reasons, Claims 31, 35, and 41-59 are *not* unpatentable over Michaeli et al in view of the combined disclosures of Greene and Park et al. Therefore, the Examiner's rejections should be REVERSED.

Respectfully submitted,

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Attachments: Claims Appendix: Pending Claims in U.S. Application Serial No. 09/441,055
Evidence Appendix
Related Proceedings Appendix

CLAIMS APPENDIX

Pending Claims in U.S. Application Serial No. 09/441,055

1. – 30. (Canceled)

31. A method for producing L-methionine which comprises culturing a recombinant *Escherichia* bacterium in a medium to produce and accumulate L-methionine in the medium, and collecting the L-methionine from the medium, wherein

the bacterium is deficient in repressor of L-methionine biosynthesis system encoded by the endogenous *metJ* gene and has L-methionine productivity,

activity of intracellular homoserine transsuccinylase encoded by the *metA* gene of a *Escherichia* bacterium is increased compared to an unmodified *Escherichia* bacterium by increasing copy number of the *metA* gene including its own promoter, or replacing the native promoter with a stronger promoter, and

the bacterium comprises at least one characteristic selected from the group consisting of:

(a) exhibits reduced activity of intracellular S-adenosylmethionine synthetase encoded by the endogenous *metK* gene as compared to an unmodified *Escherichia* bacterium;

(b) exhibits L-threonine auxotrophy;

(c) exhibits enhanced activity of intracellular cystathionine γ -synthase encoded by the *metB* gene of a *Escherichia* bacterium and enhanced activity of intracellular aspartokinase-homoserine dehydrogenase II encoded by the *metL* gene of a *Escherichia* bacterium as compared to an unmodified *Escherichia* bacterium by increasing copy number of each of the genes including their own promoters, or replacing the native promoter with a stronger promoter; and

(d) has a homoserine transsuccinylase for which concerted inhibition by L-methionine and S-adenosylmethionine is desensitized, wherein the homoserine transsuccinylase comprising the amino acid sequence of SEQ ID NO: 26 contains at least one amino acid replacement wherein said at least one amino acid replacement is independently selected from the group consisting of replacement of the amino acid residue Arg-27 with cysteine, replacement of the amino acid residue Ile-296 with serine, and replacement of the amino acid residue Pro-298 with leucine.

32. – 34. (Canceled)

35. The method according to Claim 31, wherein the bacterium is *Escherichia coli*.

36. – 40. (Canceled)

41. The method according to claim 31, wherein the bacterium comprises at least the characteristic (a).

42. The method according to claim 31, wherein the bacterium comprises at least the characteristic (b).

43. The method according to claim 31, wherein the bacterium comprises at least the characteristic (c).

44. The method according to claim 31, wherein the bacterium comprises at least the characteristic (d).

45. The method according to claim 31, wherein the bacterium comprises the characteristics (a) and (b).

46. The method according to claim 31, wherein the bacterium comprises the characteristics (a) and (c).

47. The method according to claim 31, wherein the bacterium comprises the characteristics (a) and (d).

48. The method according to claim 31, wherein the bacterium comprises the characteristics (b) and (c).

49. The method according to claim 31, wherein the bacterium comprises the characteristics (b) and (d).

50. The method according to claim 31, wherein the bacterium comprises the characteristics (c) and (d).

51. The method according to claim 31, wherein the bacterium comprises the characteristics (a), (b), and (c).

52. The method according to claim 31, wherein the bacterium comprises the characteristics (a), (b), and (d).

53. The method according to claim 31, wherein the bacterium comprises the characteristics (a), (c), and (d).

54. The method according to claim 31, wherein the bacterium comprises the characteristics (b), (c), and (d).

55. The method according to claim 31, wherein the bacterium comprises the characteristic (a), (b), (c), and (d).

56. The method according to claim 31, wherein the activity of intracellular S-adenosylmethionine synthetase is reduced due to that the bacterium has S-adenosylmethionine synthetase which contains amino acid substitution which is selected from the group consisting of replacement of the amino acid residue Ile-303 with leucine, replacement of the amino acid residue Val-185 with glutamic acid, and replacement of the amino acid residue Arg-378 and subsequent amino acid residues with the amino acid sequence of SEQ ID NO: 29, respectively in the amino acid sequence of SEQ ID NO: 18.

57. The method according to claim 41, wherein the activity of intracellular S-adenosylmethionine synthetase is reduced due to that the bacterium has S-adenosylmethionine synthetase which contains amino acid substitution which is selected from the group consisting of replacement of the amino acid residue Ile-303 with leucine,

replacement of the amino acid residue Val-185 with glutamic acid, and replacement of the amino acid residue Arg-378 and subsequent amino acid residues with the amino acid sequence of SEQ ID NO: 29, respectively in the amino acid sequence of SEQ ID NO: 18.

58. The method according to claim 31, wherein the L-threonine auxotrophy is due to deletion of the *thrBC* genes.

59. The method according to claim 42, wherein the L-threonine auxotrophy is due to deletion of the *thrBC* genes.

EVIDENCE APPENDIX

None.

RELATED PROCEEDINGS APPENDIX

None.